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CHEMICAL RESPONSES BY MARINE ORGANISMS TO
STRESS. STRESS IN HARD CLAMS FROM A POL-
LUTED ESTUARY

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Rhode Island University

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CHEMICAL RESPONSES BY MARINE ORGANISMS TO STRESS

STRESS IN HARD CLAMS FROM

A POLLUTED ESTUARY

by

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for the

WATER QUALITY OFFICE
ENVIRONMENTAL PROTECTION AGENCY

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27 Abstract (21p2) The hard clam, <u>Mercenaria mercenaria</u>, shows a general response to environmental variation. The molar ratio of free taurine to glycine in gill and mantle tissues climbs above 3, while α-amino acids and carbohydrates decrease. Subtle adjustments in the total pattern of free amino acids and fatty acids also occur, but these can be readily seen by changes in biochemical diversity and equitability. In an estuary long suffering from hydrocarbons and other agents in petroleum products and sewage, high mortality results from a culmination of natural responses superimposed on abnormal complications. The process apparently starts after a black, polymeric irritant collects in epithelial tissue and eventually occludes the renal sac. This leads, indirectly, to infestations of a parasitic polychaete that is rarely found in hard clams. A syndrome with many facets soon becomes clear, but the situation can be identified and its results predicted by simply observing the responses of taurine and glycine in stressed and normal populations. (EPA abstract)				

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SECTION I

CONCLUSIONS

1. The hard clam, Mercenaria mercenaria, a commercially valuable species in temperate estuaries along the East Coast, reacts in many ways to environmental stimuli. The magnitude of response is a measure of abnormal stressors associated with pollution. Together they can be regarded as a syndrome, which indicates the nature as well as the intensity of abnormal influences.
2. In clams from the polluted Providence River, Rhode Island, conditions of stress leading to high mortality can be recognized by the molar ratio of free taurine to glycine in mantle and gill tissue, which ranges from 5.0 to 6.6, depending upon season, as opposed to values between 2.3 and 2.9 in populations from unpolluted regions of Narragansett Bay. Furthermore, polluted clams have low amounts of total free amino acids, lower carbohydrate concentrations and slightly altered fatty-acid distributions, as compared with normal clams from the bay.
3. Patterns of distribution involving 17 or so amino acids and 19 fatty acids can be summarized with indices of biochemical dominance-diversity. These indices reveal subtle readjustments of metabolic pools as they respond to environmental change.
4. Clams held in running seawater aquaria for a year developed symptoms similar to clams from polluted waters, indicating the general nature of response to external stressors.
5. Clams from the Providence River show several morphological abnormalities: tissue blackening, calcareous ridges on the inner shell surface, a mud-blister infestation and occlusion of the kidney sac and renal tubules with a black, polymeric substance. A mechanism to account for these symptoms, as part of a larger syndrome, is postulated. The causative agent is traced back to petroleum products and other hydrocarbons discharged into the Providence River. Were it not for influxes of clam larvae from Narragansett Bay, the effects of these pollutants on the river stock would be catastrophic.

SECTION II

RECOMMENDATIONS

Routine application of the taurine/glycine index for management and control purposes should be considered by appropriate agencies. In Providence River clams, at least, this ratio constitutes a permanent "biochemical tag" that is not lost through depuration. The ratio is probably predictive of environmental abnormalities which, if allowed to persist, would lead to mass mortality.

The behavior of this index in other commercially important bivalves should be examined, especially in relation to shellfish disease and incipient weakening of invertebrate populations by the accumulation of abnormally induced reactions to environmental stressors.

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SECTION III

INTRODUCTION

Marine organisms react to their environment in a number of ways. These may be grouped as natural responses to a seasonally changing environment and as abnormal effects of artificial complications such as pollutants. The separation of natural from artificial is difficult, especially in marine organisms, so closely do they live in relation to a medium that bathes, supports, nurtures and cleanses the life processes. Consequently it is usually impossible to predict the effects of environmental stressors working in combination with one another.

To make distinctions, we need readily measured criteria intimately associated with the homeostatic process. Each departure would then represent a symptom of internal stress, but not necessarily disclose the specific environmental factors responsible for the condition. However, if various symptoms occurring together are taken as a syndrome the identification of causative factors might well be accurate. In this context, the free amino acids, fatty acids and carbohydrates of the hard clam, Mercenaria mercenaria (L.), from polluted and clean habitats of Narragansett Bay are compared.

Mortality is high in the polluted population. Observations over the last 20 years indicate that it is supported by recruitment of larvae from nearby, unpolluted waters; otherwise the polluted clams would soon disappear (G. Gray, personal communication). The symptoms involve interrelated sets of morphological abnormalities, reactions to disease and biochemical adjustments. As a syndrome they help us to predict the consequences of various factor combinations affecting the distribution and abundance of this valuable species.

METHODS

Clams were collected with a commercial (rocking chair) dredge in the West Passage of Narragansett Bay, 10.5 km from its mouth, and at Sabin Point in the Providence River, a tributary of Narragansett Bay. Salinity at the bottom usually ranged between 30-32 o/oo in the Bay and between 28-31 o/oo in the River (Hicks, 1959); in both areas temperature ranged from freezing to approximately 22C, sediment consisted of sand for the most part (McMaster, 1960) and depth was 2-5 m.

Pollution is the major environmental difference between the two stations. The Providence River receives all manner of industrial and domestic wastes, not the least of which is crude oil from tankers and storage facilities (Farrington, 1971). The Bay station, on the other hand is in an open, well-flushed region of Narragansett Bay, not far from the ocean. For convenience the River is termed "abnormally stressed" and the Bay habitat is "normal".

At each station, approximately 60 kg of clams were dredged, and the individuals ranging from 6 to 11 cm in length were saved. About 100 clams were opened and rinsed; gonadal examinations indicated that approximately 2/3 of the specimens were females. After dissecting free the stomach and foot, we homogenized the gills and mantle in distilled water and freeze dried the slurry. Dry tissue was pooled, ground to a fine powder and stored for analysis at -20C in desiccators. Clams were also maintained in wooden tanks supplied with clean, running seawater (30-32 o/oo). The tanks had no natural sediment for the clams to burrow into and detritus accumulated at a phenomenal rate ($132-162 \text{ g m}^{-2} \text{ d}^{-1}$; Blake and Jeffries, 1971). They were washed monthly, but even so shell discoloration and anaerobic conditions occurred in the sediment. This environment represents, therefore, another set of stressed conditions.

Lipids were extracted by the Bligh and Dyer procedure. Methyl esters of the fatty acids were prepared by treating the lipids first with 0.5N sodium methoxide in methanol, then with 14% boron trifluoride in methanol -- an adaptation of methods described by Luddy et al. (1960, 1968). Gas chromatographic conditions were: dual-flame ionization detectors; stainless steel columns, 1.86 x 3.2 mm, packed with 17% diethylene glycol succinate on Chromosorb W (HP), 100/120 mesh; He flow 30 ml/min; injector and detector temperatures 250C; and column temperature programmed from 165 to 190C at 4°/min after a 2 min initial period. Peaks were identified from linear relationships between log retention time and carbon-number, and they were integrated electronically. Quantitative results with NIH Fatty Acid Standards B, D and F and Hormel Institute mixture GLC 1 agreed with stated composition with a relative error less than $\pm 5\%$ for the major components (>10%). A sample chromatogram was discarded if, in replicate analysis, any component varied by more than $\pm 5\%$ of its mean value.

Free amino acids were extracted by homogenizing 0.5 g dry tissue with 15 ml water, centrifuging, washing the residue with 10 ml water, combining supernatants and adding 15 ml of 95% ethanol to precipitate proteins. After standing for 1 h, the extract was centrifuged and the supernatant was concentrated by rotary evaporation. Analysis by ion-exchange chromatography was made with a Technicon amino-acid analyzer, Model NC-1, operated according to the manufacturer's

instructions for Type B (high resolution) Chromobeads. Uncertainties averaged less than $\pm 6\%$ (Reid, 1966) for all components, significantly better than previously reported for acidic and basic components (Jeffries, 1969), probably because an aqueous extract was analyzed, rather than the supernatant from the Bligh and Dyer extract.

Carbohydrates were extracted into hot trichloroacetic acid solution (Kemp and Kits van Heijningen, 1954) and estimated colorimetrically in phenol, with anhydrous dextrose as a standard (Dubois et al., 1956).

RESULTS

Free amino acids

Distributions of free amino acids in clams collected during spring are shown in Table I. In stressed clams, the most noticeable difference is a high taurine concentration, but the total is less than in normal clams due to lower amounts of most α -amino acids -- glycine, alanine and leucine in particular. With taurine not included, total concentration in stressed clams is only 276.9 μ moles/g compared to 385.6 μ moles/g in normal clams.

Differences between the two stocks are persistent. They may become even more pronounced from April to November since the general seasonal pattern is more taurine and less α -amino acid. Consequently the two stocks can be separated at any time by the molar ratio of taurine to glycine, which ranges from 2.5 to 2.9 in normal clams and from 5.0 to 6.6 in stressed clams.

Fatty acids

During spring both stocks have very similar fatty acid patterns (Table II). The only consistent trends are comparatively low levels of 20:5 under stress and a tendency for slightly higher ratios of palmitoleic acid (16:1) to palmitic acid (16:0) in stressed clams (0.4-0.6) than is normal (0.2-0.6).

Compared with other marine invertebrates, the hard clam has a remarkably stable fatty-acid composition. At low temperatures, polyunsaturates increase in various species (Allen, 1960; Jeffries, 1970), but here this does not appear to be the case.

One peculiarity is 20:4, a minor component usually comprising less than 0.6% of the total. Under stress late in the fall, the amount may go up to 13.9%. Apparently there is a connection with 20:5, because when 20:4 increases, 20:5 falls off an equal amount, the total remaining fairly constant at about 17-19%. Whenever this

reversal occurs, 15:1 increases several times, reaching concentrations above 6%.

Seasonal ranges

Figure 1 shows the ranges of metabolites having substantially different concentrations in the two stocks. As indicated in the left and right portions of the diagram, a symptom of stress may be either a lower or higher than normal range. Glycine and carbohydrates are clearly lower in stressed than in normal clams. Alanine is also low, and the difference with normal at any one time may be great, but there is an equally large seasonal variation, and so the ranges overlap.

For the components having higher than normal concentrations under stress, only taurine is exclusive: there is no overlap into the normal.

The other components in Fig. 1 merely show tendencies toward high or low concentrations, but as persistent trends they are, nevertheless, the more prevalent and noticeable symptoms collectively describing a syndrome. Thus in stressed clams, total free amino acids and palmitic acid tend to be found in low concentrations; high values are found for palmitoleic acid and the weight ratio of palmitoleic to palmitic acids.

Preliminary separation of natural and abnormal responses

At the population level, natural responses to environmental factors fall into two major categories: seasonal and spatial. The spatial aspect is not important in this study because the physical environment at the two sampling areas is essentially the same as far as homeostatic reactions to external agents are concerned.

With the spatial aspect eliminated, natural response can be regarded as seasonal departure from values observed at a reasonable starting point (Ansell et al., 1964). For the hard clam, the starting point is April (Tables I, II): temperature has risen to the point where the animal feeds actively, reproductive products and metabolic reserves have accumulated, and with continued warming, the clam will spawn, commencing in late May and lasting through September, with peak reproductive activity in June and July (Landers, 1954). For any responding metabolite

$$\text{seasonal response} = \frac{a_t - a_o}{a_t} \cdot 100 \quad (1)$$

where a_o is the concentration in normal clams during April and a_t is the value observed in this stock at some time later in the year.

Abnormal effect in stressed clams is also expressed relative to normal

$$\text{abnormal effect} = \frac{b_t - a_t}{a_t} \cdot 100 \quad (2)$$

where b_t is the concentration of a component in stressed clams.

Net response is the sum of natural (seasonal) response and abnormal effect, so adding (1) and (2), we get

$$\text{net response} = \frac{b_t - a_o}{a_t} \cdot 100 \quad (3)$$

The more important results are shown in Fig. 2, along with responses of stressed clams held for one year in laboratory tanks supplied with clean seawater. The latter are related to normal clams which were collected at the same time (a_o) and held in identical tanks (a_t). The course of net response is cumulative, except for taurine and glycine, which appear to mirror image each other. Alanine exhibits the largest relative change.

Laboratory conditions

A progressive reaction to abnormalities imposed in the laboratory is also apparent in Table III, which shows three measurements over 12 months on transplanted clams. Taurine increases from 212.6 to 264.1 $\mu\text{moles/g}$ in clams from clean water, but this is still far less than the 315.4 $\mu\text{moles/g}$ found in stressed transplants held for the same period. Conversely, glycine in the normal transplants decreases from 84.2 to 54.3 $\mu\text{moles/g}$, which is probably near the lower level reached by this amino acid in *Mercenaria*. Alanine also decreases, approaching a minimum of approximately 31 $\mu\text{moles/g}$ after a year in the laboratory. From this it is concluded that glycine and alanine reach minima in the laboratory, regardless of the clam's environmental history. Taurine, however, is exceptional: first because it increases under stress, and second because there is an additive effect involving the responses to abnormalities originating prior to transplanting. This latter abnormality appears to be associated with a mud-blister infection that is unique to the stressed clams.

DISCUSSION

Environmental conditions in the Providence River (the stressed habitat) have obviously deteriorated over the past 50 years or so. Farrington (1971) reports high concentrations of hydrocarbons in river sediment that he traces to petroleum and sewage. Upon being released into the river, hydrocarbons undergo a series of quick reactions, leaving behind cyclic alkanes and aromatic compounds that degrade slowly and become incorporated into sediments and molluscs. This complex mixture appears in a gas chromatogram as a broad hump, or envelope, beneath the n-alkane peaks (Blumer, et al., 1970).

A black, tar-like discoloration is found in many clams from the Providence River. Much of it is resistant to lipid solvents, and it lasts for at least a year after the clams have been transplanted to a clean, natural habitat or the laboratory tanks used here. We suspect that the discoloration is a polymer derived from petroleum products and hydrocarbons in other pollutants.

Amoebocytes are able to remove the pollutant, perhaps directly from solution (pinocytosis) or by phagocytosis if it is in particulate form. Masses of black amoebocytes are seen in histological sections of mantle and kidney tissues from stressed clams; in normal clams these cells are far less numerous, especially in the kidney, and they are not blackened (Paul Yevich, personal communication). This black material collects in the renal sac, in quantities that would appear to plug the tubules and interfere with normal kidney function.

There is also a high incidence (5-10%) of a mud-blister infestation associated with the discoloration, which S. Feng (personal communication) indicates is Polydora sp., a common parasite in the American oyster (Crassostrea virginica) but rare in molluscs that burrow into sediments. This may indicate that clams in the river emerge from sediments -- perhaps in response to irritants, low oxygen and other conditions associated with pollution -- exposing themselves to attack by Polydora larvae, which are abundant in Narragansett Bay during summer (Jeffries, 1964). In addition discolored clams often have a calcareous ridge extending longitudinally on the inner surface of the shell. Ridges of this nature are formed by the mantle when it retracts in response to an irritant.

Thus we see an interrelation of natural and abnormal factors in the Providence River, culminating in conditions that the clam cannot endure. The shells gape open in about 1% of the individuals, exposing their dead and decomposing soft parts. Mortality is most noticeable among larger specimens approximately 11 cm long. Indeed empty shells

larger than this are rare, and, because shellfishing has been prohibited in the river for years, small size would indicate an early death, compared to Narragansett Bay, where living clams 15 cm and longer are often found.

Young clams are abundant in the river, but it is obvious that when 1% of the adults dies every day or so during the warm months, the population might disappear in a short time. A year after the investigation, only a few clams could be found in a once productive portion of the sampling area.

Mass mortality is a common phenomenon in the paleontological record (Brongersma-Sanders, 1957), which can often be attributed to the cumulative effects of a persistent environmental change. The question facing us today is: how can we separate responses to normal environmental variation from the effects of cataclysmic natural processes and incipient abnormalities such as pollution?

In the situation examined here, abnormal reactions are superimposed on natural seasonal variation, so whatever the natural burden on homeostasis may be, environmental abnormalities serve to make it even worse (Fig. 2). There is not a cancelling effect whereby normal variation in one direction offsets abnormal variation in the opposite direction.

Since the effects of natural and artificial stressors differ only in magnitude, we can identify a generalized stress syndrome. When a certain severity is reached, the population dies. In its simplest form, this syndrome can be recognized by taurine/glycine molar ratios greater than 3.

A similar pattern in the American oyster infected with Minchinia nelsoni and Bucephalus sp. has been described by Feng et al. (1970). From his Table 1, ratios of taurine to glycine in the hemolymph can be calculated: the ratio in normal oysters ranges from 0.5 to 2.0 with a mean of 1.2; in infected oysters the range is 1.3 to 9.1, the mean is 4.0 and the concentration of total free amino acids is low. Feng proposes a compensatory mechanism: keto acids are converted to amino acids that have been depleted by parasites; meanwhile an elevated taurine level helps the host to maintain osmotic balance with the external environment.

Polydora, however, is not a true parasite in the sense that a nutritional benefit is derived from the host's metabolism. But the average taurine/glycine ratio in an infected clam is even higher than in a diseased oyster, and uninfected clams held in the laboratory for one year have a 4.9 average value. It follows that this ratio indicates a general reaction by the clam to trauma, laboratory conditions, pollutants and seasonal variations in the environment. Moreover the magnitude points to causative agents: values between 3 and 5 result from physical-chemical abnormalities in the environment; anything greater than 5 indicates disease. The extreme may also disclose a

severely weakened condition that made it possible for the disease to become prevalent in the first place.

Other symptoms include group responses of entire classes of metabolites, and it is proposed that dominance-diversity relationships are an easy and sensitive way of examining these complex patterns.

Biochemical dominance-diversity

Awapara (1962) states that free amino acid patterns are "finger prints" representing a picture of an organism's metabolic activities -- a balance between formation and utilization. Fingerprints are difficult to describe, however, especially when the whole picture is considered. Statistical procedures have their place (Jeffries, 1969), but we can also apply the diversity concepts used by ecologists to compare species distributions and community structure.

Most formulations of diversity take into account how many species are present and their individual abundances. The more species there are and the more numerically equal their individual abundances are, the higher the diversity. A fundamental problem exists, however, because the number of species found in a sample increases with sample size, and as sample size is increased common species become all the more common (Hairston, 1969). Although this is not nearly as serious with the Shannon-Wiener and rarefaction methods as it is for the logarithmic series (Sanders, 1968), there are obvious difficulties when one compares the results of several investigators. Thus it is believed that biochemical diversity has a place in ecology: the major chemical species, such as amino acids and fatty acids, are ubiquitous monomers (Morowitz, 1968); these substances are readily identified and measured in relation to independent standards; and it is reasonable to propose the idea that there are chemical analogs of community structure.

With a fixed number of biochemical "species", all we need to be concerned with is the proportion of each -- the evenness component of ecological species diversity. Dominance, the opposite of evenness, is easier to envision than diversity and it is readily calculated (Jeffries, 1970). The index of biochemical dominance, D_{mp} , for n-many constituents indicates the departure from a distribution in which each component has the same relative concentration. The reciprocal of dominance $\times 100$ is the index of biochemical diversity, D_{vn} , which usually gives values directly related to diversity in the usual sense, e.g. $H(S)$, the Shannon-Wiener index.

As shown in Table IV, biochemical dominance of free amino acids is greater in stressed than in normal clams, and it increases seasonally, with maxima found in laboratory transplants (Table IV). These

patterns simply reflect an increase in taurine and a decrease in α -amino acids, both seasonally and abnormally induced.

Conversely dominance among the fatty acids is greater in normal than in stressed clams; spring and fall values for each stock are about equal and there is a considerable drop in the transplants.

Equitability (ϵ), or evenness of the distribution related to a random model (Lloyd and Ghelardi, 1964), also reveals the extent to which taurine dominates the free amino acids (Table V). With taurine included, ϵ is 0.60 in stressed clams and 0.75 in normal (April), but with taurine eliminated from the calculations, the corresponding values are 1.07 and 0.97. A value near 1 means that concentrations within the α -amino acid pool are randomly distributed, a situation analogous to that predicted by MacArthur's model for random, nonoverlapping niches among the species of a community. In autumn, however, ϵ drops well below unity, reaching 0.80 in normal clams and 0.89 under stress. Fatty acids distributions behave oppositely: stressed is greater than normal, and the trend is toward higher values seasonally and in the laboratory. Under these conditions, the near approximation to a random distribution is remarkable.

For practical purposes, the taurine/glycine ratio may be sufficient to recognize abnormalities in the hard clam. In view of our incomplete understanding of the many ways that marine organisms react to their surroundings, it does seem useful to keep in mind the various symptoms listed in Table VI. The manner in which these symptoms are interrelated, their mechanisms and regional variations offer many opportunities for further investigation.

SECTION IV

Table I

Free amino acid composition of Mercenaria mercenaria
 from normal and stressed habitats in Narragansett Bay,
 April 1969, μ moles/g dry wt.

Amino acid	Normal	Stressed
Taurine	205.4	247.5
Glycine	81.1	37.6
Alanine	76.3	52.2
Glutamic	54.9	43.4
Aspartic	27.0	28.8
Leucine	23.3	16.1
Threonine	21.3	17.7
Valine	16.2	12.0
Isoleucine	14.2	9.6
Serine	12.6	10.2
Phenylalanine	12.4	8.0
Arginine	12.2	13.4
Tyrosine	11.6	7.9
Cysteic	7.3	8.4
Histidine	6.5	4.0
Lysine	4.8	5.6
Methionine	3.9	2.0
Proline	+	+
Ornithine	+	+
Total	591.0	524.4

Table II

Fatty acid composition of Mercenaria mercenaria from
normal and stressed habitats in Narragansett Bay,
April 1969; percent of total fatty-acid methyl esters.

Fatty acid ^a	Normal	Stressed
14:0	1.0	1.0
15:0	1.3	1.3
15:1	1.2	3.0
16:0	12.2	12.8
16:1	7.0	6.0
16:2	0.5	1.0
17:0	4.2	4.6
17:1	0.5	2.0
18:0	6.5	6.2
18:1	5.9	6.1
18:2	0.3	0.7
19:1	0.2	0.1
20:0 ^b	0.6	0.5
20:1 ^c	9.4	8.4
20:2	1.4	1.1
20:4	0.5	0.5
20:5	19.5	16.7
22:0 ^d	3.6	4.6
22:4 ^e	1.1	1.3
22:5	3.4	2.5
22:6	16.3	15.4
Other C ₂₀₋₂₂ ^f	3.3	4.1

^a shorthand designation for number C atoms:number double bonds

^b includes 18:3

^c includes 18:4

^d includes 22:1(?)

^e includes 24:1

^f 21:4, 21:5, 23:1(?)

Table III

Free amino acid concentrations in Mercenaria mercenaria collected in November 1968 from normal and stressed habitats and held in tanks supplied with clean, bay water. In parentheses, corresponding values for natural (field) populations are given. $\mu\text{moles/g dry wt.}$

	<u>Normal</u>		<u>Stressed</u>	
Taurine				
Nov.	212.6		-- a	
July	245.2	(216.7)	277.2	(290.2)
Nov.	264.1	(221.3)	315.4	(262.9)
Glycine				
Nov.	84.2		-- a	
July	73.6	(95.3)	57.8	(52.6)
Nov.	54.3	(76.4)	54.2	(52.7)
Alanine				
Nov.	55.4		-- a	
July	49.0	(57.2)	48.5	(37.3)
Nov.	34.8	(44.6)	31.0	(34.3)

^a data not available for November 1968.

Table IV

Biochemical dominance of free amino acids (D_{m1}) is greater in hard clams from stressed than normal habitats; increases are apparent seasonally and after transplantation to laboratory tanks; values in parentheses are the same data recalculated without taurine (D_{m16}). Among the fatty acids, D_{m19} is greater in normal than stressed clams and the transplants are uniformly low; in these calculations, 16:2 and 17:1 have been combined and 19:1 eliminated (cf. Table 2).

	Normal	Stressed
Amino acids		
April	29.1 (23.8)	31.5 (21.7)
November	34.4 (28.3)	35.7 (26.3)
Transplants	36.3 (28.0)	37.6 (28.2)
Fatty acids		
April	27.5	25.0
November	27.3	24.2
Transplants	23.5	23.5

Table V

Equitability (ϵ) relationships of free amino acids in normal and stressed clams depend on whether taurine is included in the calculation or eliminated (values in parentheses). With taurine included, normal is greater than stressed, but if taurine is eliminated, ϵ then taking into account only the α -amino acids, stressed is greater than normal. Equitability among the free amino acids decreases seasonally and when clams are brought into the laboratory (transplants); the opposite trend is indicated for fatty acids.

	Normal	Stressed
Amino acids		
April	0.75 (0.97)	0.60 (1.07)
November	0.54 (0.80)	0.45 (0.89)
Transplants	0.44 (0.82)	0.38 (0.81)
Fatty acids		
April	0.86	0.94
November	0.87	0.97
Transplants	0.99	0.99

Table VI

Summary of a stress syndrome in Mercenaria mercenaria:

salinity 28-32 o/oo, temperature 14-22 C; April-

November, 1969; Narragansett Bay.

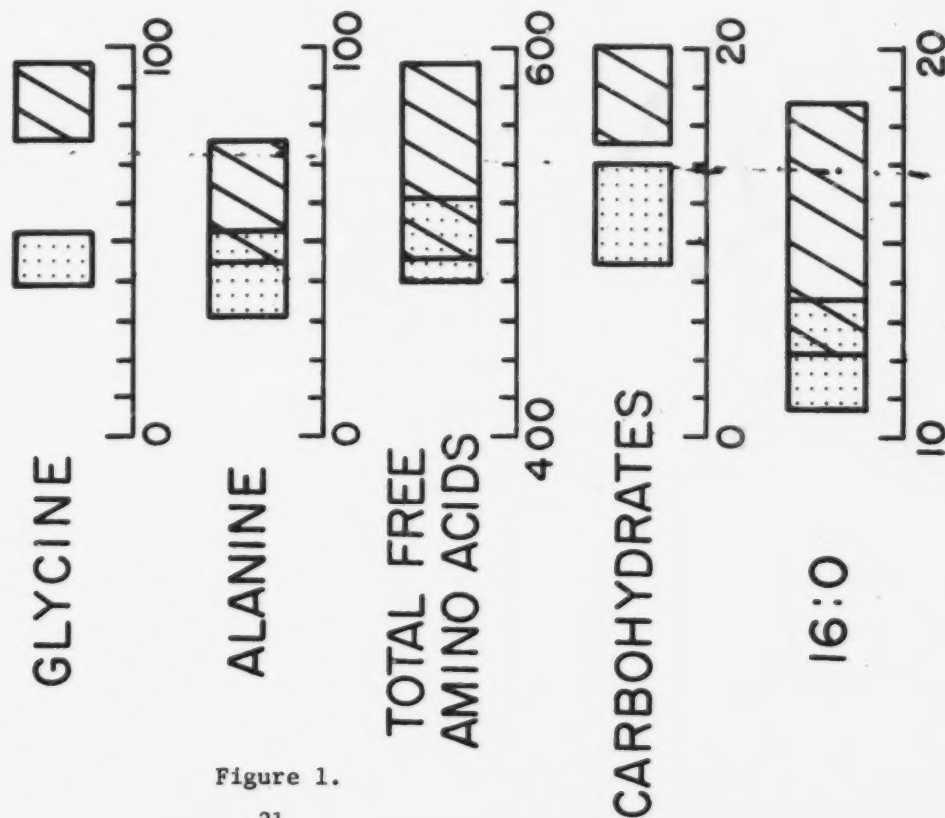
Variable	Normal	Symptomatic
<u>Composition</u>		
Taurine	<230, seasonal	>240, may exceed 300 in
μmoles/g	increase	laboratory
Glycine	>70	<60
μmoles/g		
Taurine/glycine	2.3-2.9	>3.0; ca. 4.9 in labora-
molar ratio		tory; 5.0-6.6 if infected
Alanine	seasonal decrease	seasonal decrease
μmoles/g	80 to 40	60 to 30
Total free amino	seasonal decrease	seasonal decrease
acids μmoles	590 to 490	530 to 480
Total carbohydrates	>15	<15
%		
16:0 %	generally >13	generally <13
16:1/16:0	0.2 - 0.6	0.5 - 0.6
wt ratio		
20:4 %	generally <1	may exceed 9, associated
		with a drop of 20:5 from
		about 20 to 6
<u>Dominance-diversity</u>		
D _m amino acids	seasonal decrease	seasonal decrease
taurine included (D _{m17})	29 - 35	31 - 36; max 38(laboratory)
no taurine (D _{m16})	23 - 28	21 - 26; max 28(laboratory)
ε amino acids		
taurine included	0.5 - 0.8	0.4 - 0.6
no taurine	0.8 - 1.0	0.8 - 1.1
D _{m19} fatty acids	27 - 31	25 - 29; 23.5 in laboratory
ε fatty acids	0.8 - 1.0	0.9 - 1.0; 1.0 in laboratory

Table VI (continued)

Variable	Normal	Symptomatic
<u>Gross anatomy</u>		
<u>Polydora</u> sp.	absent	infected
color of tissues	white-yellow	grey-black
inner surface of		
shell	smooth	calcareous ridges
renal sac	open	may be occluded with a tar-like deposit

STRESS SYMPTOM

LOW Concentration



HIGH Concentration

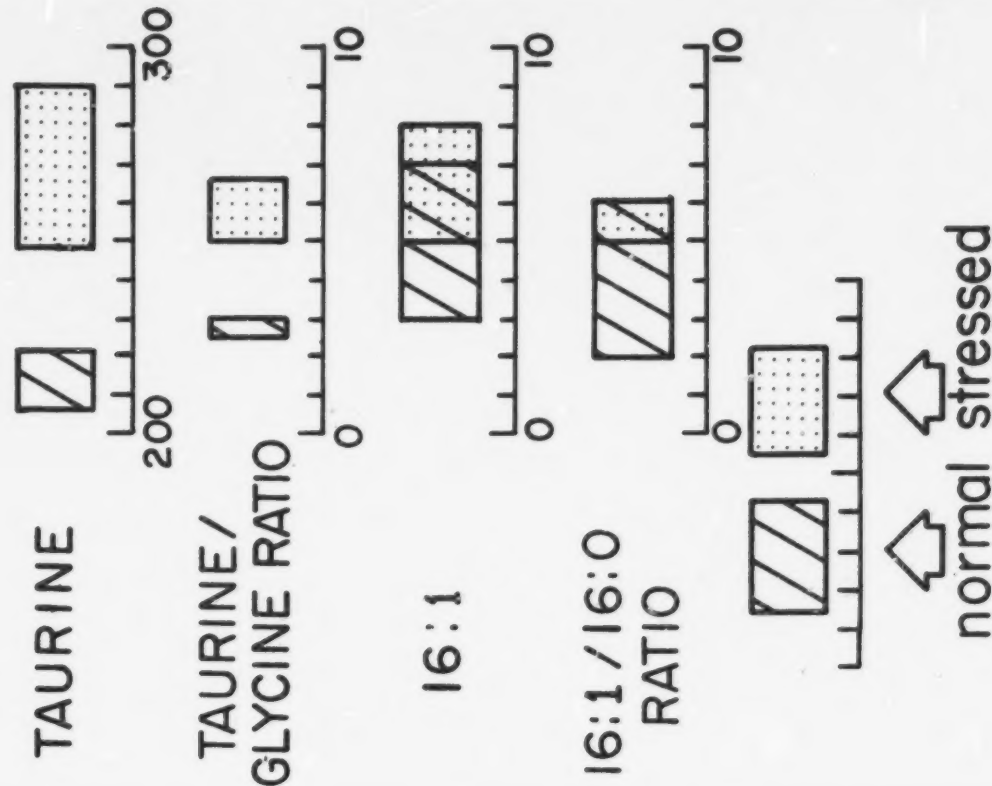


Figure 1.

RELATIVE VARIATION %

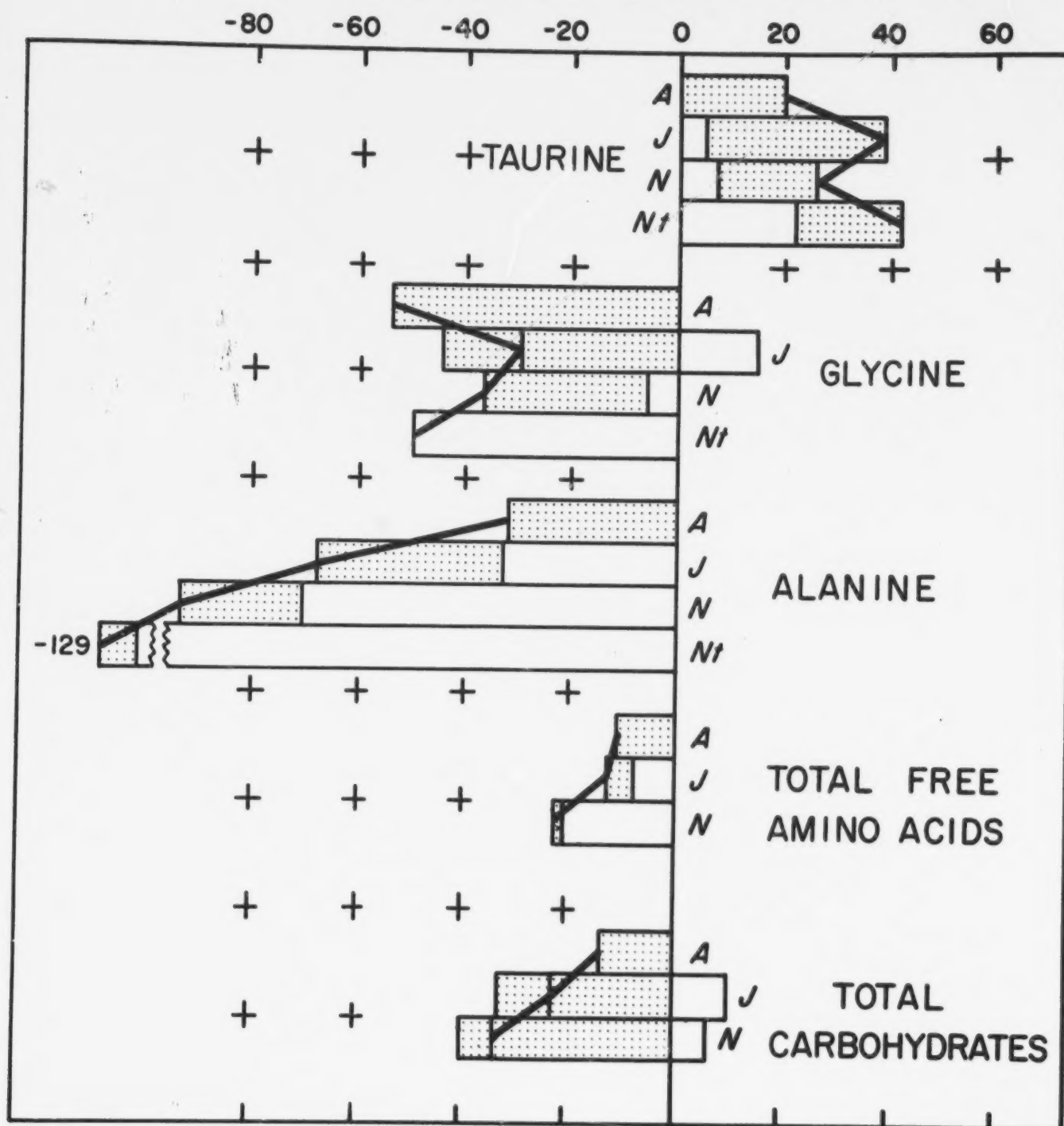


Figure 2.

SECTION VI

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Dr. Donald K. Phelps, National Marine Water Quality Laboratory (EPA) provided suggestions and observations on physical environmental variables. Mr. Paul Yevich of the same laboratory kindly made available photographs and interpretations of histological changes taking place in Providence River clams. Miss Gale Nigrelli and Miss Mary Bannister assisted in the laboratory. This investigation was supported by the Environmental Protection Agency, with Dr. Gilles LaRoche as Grant Project Officer. For all this help, advice and support I acknowledge sincere thanks.

SECTION VII

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SECTION VIII

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SUPPORTED BY GRANT 18050-DTX

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